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## SHORT REPORT

# Regulation of peripheral classical and non-classical monocytes on infliximab treatment in patients with rheumatoid arthritis and ankylosing spondylitis

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## ABSTRACT

**Objective:** To investigate the regulatory effect of tumour necrosis factor (TNF) blockade with infliximab on the distribution of peripheral blood monocyte subpopulations in patients with rheumatoid arthritis (RA) and ankylosing spondylitis (AS).

**Methods:** Purified CD11b+CD14+ monocytes from 5 patients with RA and 5 AS were analysed ex vivo before and after infliximab treatment by flow cytometry for CD16, CD163, CD11b, C-C chemokine receptor type 2 (CCR2) and CXC chemokine receptor 4 (CXCR4) at baseline and at days 2, 14, 84 and 168 after the first infliximab administration. Serum levels of the stromal cell-derived factor (SDF)-1 and monocyte chemotactic peptide (MCP)-1 at different time points were measured in either patient group before and on infliximab treatment.

**Results:** Anti-TNF treatment with infliximab led to a significant increase of circulating CD11b+ non-classical and a concomitantly decrease of CD11b+ classical monocytes, to a decline in SDF-1 levels and reduced expression of CCR2 and CXCR4 on non-classical monocyte subpopulation.

**Conclusions:** Our study shows, that TNF $\alpha$  blockade by infliximab resulted in a dichotomy of the regulation of classical and non-classical monocytes that might have substantial impact on inhibition of osteoclastogenesis and of subsequent juxta-articular bone destruction and systemic bone loss in RA and AS.

## INTRODUCTION

Tumour necrosis factor (TNF) inhibition leads to a reduction of soluble cell adhesion molecules, chemokines and their receptors in patients with rheumatoid arthritis (RA)<sup>1,2</sup> or ankylosing spondylitis (AS).<sup>3</sup> In Crohn's disease, infliximab therapy has been found to increase the frequency of circulating CD14+CD16+ monocytes.<sup>4</sup> These non-classical

## Key questions

### What is already known about this subject?

- Tumour necrosis factor (TNF) inhibition leads to a reduced bone resorbing activity of osteoclast precursor cells in patients with rheumatoid arthritis (RA) or ankylosing spondylitis (AS).

### What does this study add?

- We demonstrated that anti-TNF $\alpha$  treatment response on infliximab in patients with RA and AS is associated with a rapid decline in the number of circulating classical monocytes, reduced expression of CXC chemokine receptor 4, C-C chemokine receptor type 2 and circulating stromal cell-derived factor-1.

### How might this impact on clinical practice?

- Provide a mechanistic understanding of the anti-osteoclastogenic and bone preserving effect of anti-TNF $\alpha$  treatment on infliximab in patients with RA and AS.

monocytes have been found to produce large amounts of TNF<sup>5</sup> but are not able to differentiate into osteoclasts,<sup>6</sup> whereas classical monocytes (CD14+CD16<sup>−</sup>) are mainly producers of interleukin-10<sup>6</sup> and are able to differentiate into osteoclasts.<sup>6</sup> Differential therapeutic regulation of these monocyte subsets may explain the bone-sparing effect of TNF inhibitors. This has yet not been elucidated ex vivo.

## METHODS

Patients with RA according to the ACR 1987 revised criteria<sup>7</sup> and patients with AS according to the New York criteria<sup>8</sup> were included in the present study. Patients who fulfilled the EULAR<sup>9</sup> and ASAS<sup>10</sup> response criteria to treatment with infliximab after 3 months were analysed based on data sets obtained at

all defined time points during the entire study period. Concomitant medications such as corticosteroids >7.5 mg prednisone equivalent daily, bisphosphonates, antiepileptics, heparin, oestrogens, testosterone, thiazids and ACE inhibitors were exclusion criteria. Repetitive blood samples from patients were obtained by venipuncture. To elucidate changes of monocyte subsets, positively selected (CD14 microbeads; Miltenyi Biotec, Bergisch-Gladbach, Germany) CD14<sup>+</sup> peripheral blood monocytes were measured for CD14, CD16, CD163, CD11b, C-C chemokine receptor type 2 (CCR2) and CXCR4 chemokine receptor 4 (CXCR4; antibodies all from BD Pharmingen, San Diego, California, USA) expression by FACS at baseline and days 2, 14, 84 and 168 after the first infliximab administration.<sup>11</sup> Infliximab was administered intravenously at baseline and after 2, 4, 6, 12 and 24 weeks (3 mg/kg in patients with RA and 5 mg/kg in patients with AS)<sup>11</sup> and adjusted according to clinical response thereafter. Blood was examined for changes in monocytes subsets, circulating levels of stromal cell-derived factor (SDF)-1 (Quantikine Kit (R&D Systems, Minneapolis, Minnesota, USA) and of monocyte chemotactic peptide (MCP)-1 (BD Pharmingen) which were determined by ELISA. The minimum detectable dose of SDF-1 was 18 pg/mL and of MCP-1 19 pg/mL.

Because of only 10 patients tested we used repeated measures analysis of variance and post hoc Bonferroni's Multiple Comparison Test (95% CIs), significant results ( $p < 0.05$ ) are depicted with an asterisk. Exact  $p$  values were calculated by one-tailed paired  $t$  test. Analysis and graphs were performed using prism 5 (GraphPad V.5.0).

## RESULTS

### Patient's characteristics prior to infliximab treatment

Baseline characteristics of the 10 patients included in the present study are shown in [table 1](#). Based on the strict exclusion criteria for co-medication and for definition of active disease and of treatment response, the number of finally included patients was only 10. As anticipated, the majority of RA patients were postmenopausal middle-aged females, whereas the AS group included only younger and middle-aged males. Therapy with symptomatic and/or disease-modifying antirheumatic drugs remained unchanged during the study period. Two patients with AS had no co-medication at study entry and during the entire study period. Before the first infliximab infusion, all patients had active disease according to the Disease Activity Score 28 (DAS28)<sup>9</sup> and Bath Ankylosing Spondylitis Disease Activity Index (BASDAI).<sup>12</sup>

### Increase of non-classical and decrease of classical monocytes during infliximab treatment

Throughout the study period, the number of CD11b<sup>+</sup>CD14<sup>+</sup> monocytes remained constant in either patient population (range 84.8–98.6%). However, the monocyte

**Table 1** Patient's baseline characteristics

	RA (n=5)	AS (n=5)
Female (n)	4	0
Male (n)	1	5
Age, years (range)	48.6 (39–59)	37.2 (28–47)
Disease duration, years (range)	5.2 (1.5–11)	5.48 (3–10)
DAS28, <sup>9</sup> mean (range)	4.93 (3.54–5.94)	–
BASDAI, <sup>12</sup> mean (range)	–	4.27 (3.83–4.6)
ESR, mm/h, mean (range)	21 (10–41)	38 (12–71)
CRP, mg/L, mean (range)	6 (3–17)	27 (3–49)
RF+ (%)	80	0
aCCP+ (%)	80	0
HLA-B27+ (%)	0	100
Prednisone 7.5 mg/d (n)	3	0
Methotrexate 15 mg/w (n)	2	0
Leflunomide 20 mg/d (n)	2	0
NSAIDs (n)	3	3

AS, ankylosing spondylitis; BASDAI, Bath Ankylosing Spondylitis Disease Activity Index; CRP, C reactive protein; DAS28, Disease Activity Score 28; RA, rheumatoid arthritis.

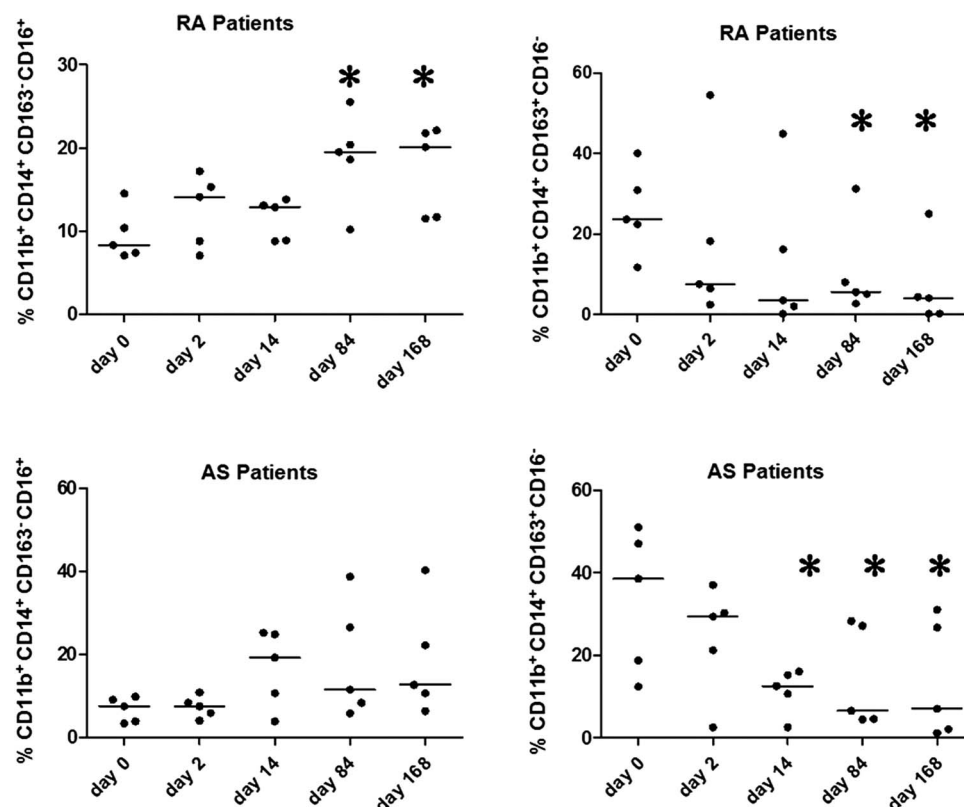
subpopulations of interest exhibited a contrasting regulation pattern on infliximab treatment both in patients with RA and AS ([figure 1](#)). Whereas the non-classical (CD11b<sup>+</sup>CD14<sup>+</sup>CD163<sup>+</sup>CD16<sup>+</sup>) subpopulation increased over time (for RA: range of 7.1% up to 25.5%; range of  $p$  value compared with baseline from 0.09 to 0.013; for AS: range of 3.4% up to 40.3%; range of  $p$  value compared with baseline from 0.34 to 0.045), the classical (CD11b<sup>+</sup>CD14<sup>+</sup>CD163<sup>+</sup>CD16<sup>–</sup>) subpopulation decreased (for RA: range of 40.1% down to 0.2%, range of  $p$  value compared with baseline from 0.14 to 0.003; for AS: range 47% down to 1.1%, range of  $p$  value from 0.023 to 0.002).

### Downregulation of circulating ligand levels (SDF-1 and MCP-1) and expression of their receptors (CCR2 and CXCR4) on infliximab treatment

Serum levels of the corresponding cytokines (SDF-1 and MCP-1) at different time points were measured concomitantly in either patient group before and on infliximab treatment. We found a reduction of circulating SDF-1 levels over time in patients with RA and AS and less significant also of MCP-1 levels after 6 months in patients with RA only ([figure 2A](#)). In addition, we observed a significant and rapid downregulation of the percentage of CXCR4<sup>+</sup> and CCR2<sup>+</sup> classical monocytes 2 days, 2 weeks, 3 months and 6 months after initiation of infliximab treatment compared with baseline values in patients with RA ( $p$  values of 0.026, 0.037, 0.028 and 0.02;  $p$  values of 0.046, 0.029, 0.029 and 0.029, respectively; [figure 2B](#)).

## DISCUSSION

In the present study, we demonstrated that in patients with RA and AS anti-TNF $\alpha$  treatment response on



**Figure 1** Changes in non-classical (CD11b+CD14+CD163–CD16+) and classical (CD11b+CD14+CD163+CD16–) monocytes over time in patients with rheumatoid arthritis (RA) and ankylosing spondylitis (AS). Data are shown as percentage of CD11b+CD14+ cells and median (\*p<0.05).

infliximab is associated with a rapid decline in the number of circulating classical (CD11b+CD14+CD163+CD16–) monocytes with reduced expression of CXCR4, CCR2 and decreased levels of circulating SDF-1. In contrast, we observed a constant accumulation of circulating non-classical (CD11b+CD14+CD163–CD16+) monocytes. Only in patients with RA we found also a decline of circulating MCP-1. Thus, our study shows, that successful TNF $\alpha$  blockade by infliximab resulted in a dichotomy in the regulation of monocytes subpopulations that may have a substantial impact on reduced bone resorbing activity of osteoclast precursor cells (OPCs) that we have observed in a previous study.<sup>11</sup>

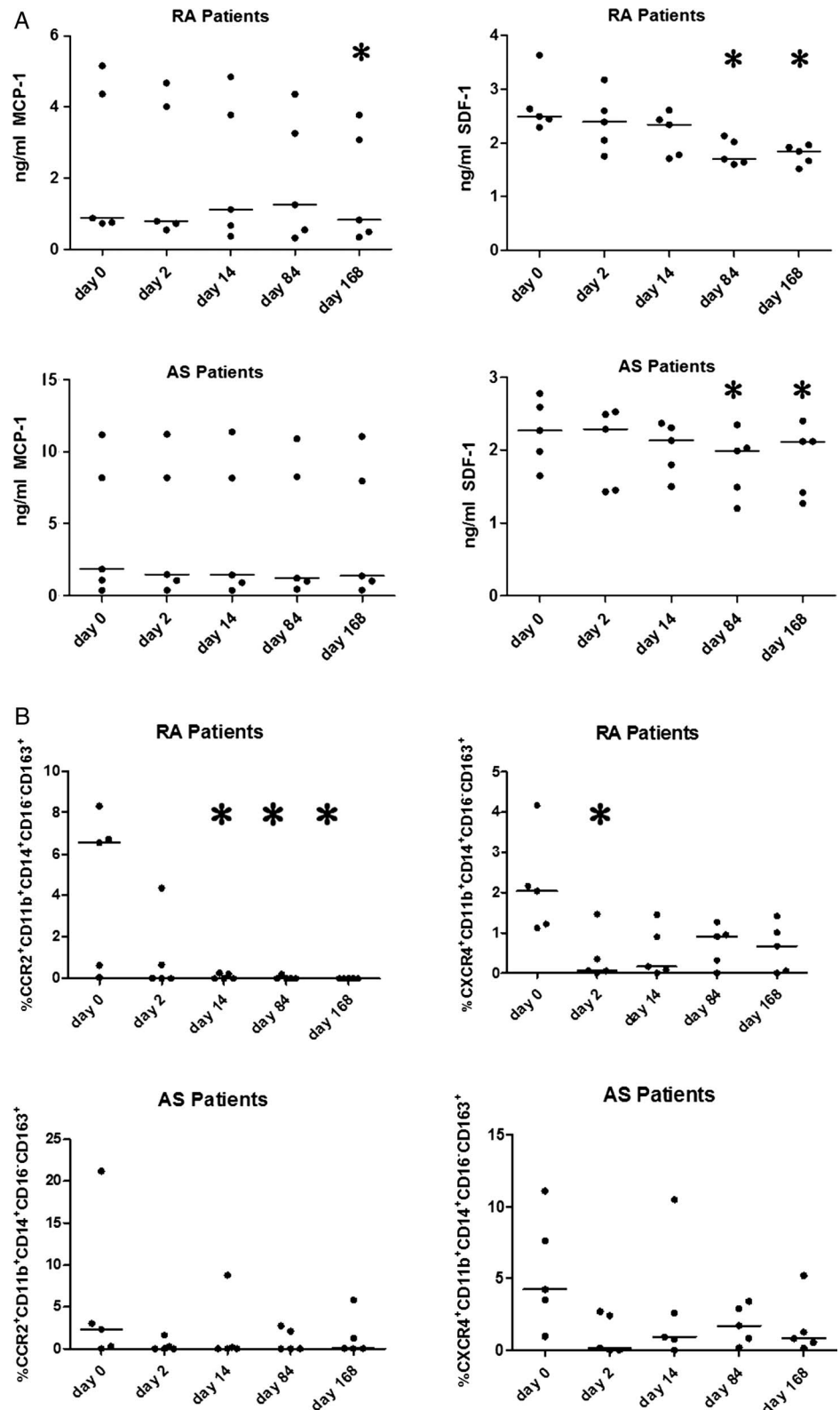
In order to get a deeper insight into the nature of potential inhibitory mechanisms, we focused on factors influencing the recruitment and migration behaviour of these cells. We first demonstrated that besides reducing the pool of circulating classical CD163+CD16– monocytes, infliximab treatment did also reduce the amount of CD163+CD16– cells bearing the chemokine receptors CXCR4 and CCR2 and concomitantly reduced the levels of corresponding circulating chemokines. These findings may suggest that besides promotion of apoptosis of monocytes as described previously<sup>13</sup> a specific inhibition of recruitment and/or trafficking of classical monocytes and of subsequent osteoclasts development at inflammatory sites might contribute to the inhibition of bone

resorption by infliximab in TNF $\alpha$ -mediated chronic inflammatory processes. Regarding the inhibition of recruitment of OPCs from bone marrow corresponding mechanisms have been already described previously in patients with psoriatic arthritis.<sup>14</sup> The inhibition of SDF-1 secretion, that is, from activated synovial fibroblasts and reduced CXCR4 expression on circulating classical monocytes<sup>15</sup> might finally inhibit recruitment, directed trafficking into inflamed peripheral tissues and subsequent osteoclastogenesis.

The peripheral pooling of CD16+ monocytes and of Th1 cells<sup>16</sup> on infliximab treatment might reflect an anti-inflammatory effect which is hypothetically mediated by the downregulation of expression of adhesion molecules on endothelial cells and by subsequent inhibition of transmigration of inflammatory cells through postcapillary venules into inflammatory sites.<sup>17, 18</sup> Our finding of a reduced recruitment of CD163+ monocytes during infliximab treatment is also in good accordance with previous results demonstrating a reduced influx of CD163+ monocytes into the synovial compartment of patients with RA and spondyloarthritis,<sup>19</sup> a reduced osteoclastogenesis in the synovial compartment of patients with psoriatic arthritis<sup>15</sup> and with the reversal of the increased frequency of CD11b<sup>high</sup> osteoclast precursors and enhanced osteoclastogenesis in TNF-tg mice on anti-TNF $\alpha$  treatment in vivo.<sup>20</sup>

**Figure 2** (A) Reduction of circulating stromal cell-derived factor (SDF)-1 levels over time in patients with rheumatoid arthritis (RA) and ankylosing spondylitis (AS) and less significant also of monocyte chemotactic peptide (MCP)-1 levels after 6 months in patients with RA only. Data are shown as absolute values and median (\* $p < 0.05$ ).

(B) Downregulation of the percentage of CXCR4+ and CCR2+ classical monocytes 2 days, 2 weeks, 3 months and 6 months after initiation of infliximab treatment compared with baseline values in patients with RA. Data are shown as percentage of CD11b+CD14+CD163+CD16- cells and median (\* $p < 0.05$ ).



A limitation of our study is the low numbers of included patients that could be analysed longitudinally.

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**Contributors** DA, MS, WH and PMV were involved in conception and design, analysis and interpretation of data. RK and DB took part in

acquisition of data. DA, MS and WH took part in drafting the article. MS, WH and PMV took part in revising it critically for important intellectual content. DA, MS and WH took part in final approval of the version to be published.

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**Competing interests** None declared.



**Ethics approval** The study design was approved by the ethics committee of the canton of Bern and a written informed consent was obtained from each patient before study enrolment.

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**Data sharing statement** No additional data are available.

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